THE SOCIETY OF AMERICAN BACTERIOLOGISTS. III

PATHOLOGIC BACTERIOLOGY

Cultivation and Differentiation of Fusiform Bacilli: CHARLES KRUMWIEDE, Jr., and JOSEPHINE PRATT, Research Laboratory, Department of Health, New York City.

Isolation: Dilutions of the original material are made in a series of tubes of ascitic fluid or horse serum. To these is added fluid agar and they are then poured in the covers of petri dishes. While the agar is still fluid the lower part of the petri dish is laid on the agar, giving a layer of agar between the two parts of the dish. After forty-eight to seventy-two hours the upper glass is separated from the agar and the distinctive colonies fished. The colony is characterized by the thread-like outgrowths from one or both sides of the colony. Cultivation: A semi-solid medium employing stab inoculation is most convenient for preservation of cultures. The puncture closes after inoculation and subinoculations are easily performed, due to the softness of the medium. Aerobic contamination is quickly noted. The medium is prepared as follows:

Agar	10 gms.	2 parts
Gelatin	80 gms.	
Veal broth, 2 per cent. peptone, no salt	1,000 c.c.	
Horse serum or ascitic fluid		1 part

Horse serum has given more uniform results than ascitic fluid. Although there is a difference in various strains in their ability to grow on simple media, serum containing media are necessary for surety of cultivation of all strains.

Source and Number of Cultures being Studied

Noma	2 strains	Total 15
Vincent's angina	5 strains	
Spongy bleeding gums	1 strain	
Pyrrhœa	2 strains	
Chronic otitic media foul discharge	3 strains	
Carious teeth	1 strain	
Ulceration of tongue	1 strain	

Morphology and Cultural Differentiation: The typical bacillus is more or less pointed. In cultures they are extremely pleomorphic, filaments and wavy forms simulating spirochetes being found. No morphological differentiation has been made. Sugar fermentations show some differences, but these differences show no relation to the source of the culture. Pathogenicity: Abscesses can be produced under the thin skin covering the cartilage of the ear of rabbits. Relation to Spirochetes: Spirochetal-like forms can be found especially in fluid media. The relation of these to the spirochetes in the original material has not been sufficiently studied.

The Morphology of Cultural Amebas: ANNA WESSELS WILLIAMS, Research Laboratory, Health Department, City of New York.

The paper was a report of the studies on cultural amebas grown under conditions as nearly as possible like those of the habitat from which the amebas were obtained. "Ameba 11,524," obtained originally by Musgrave and Clegg from the stools of a case of human amebic dysentery, when grown on fresh brain tissue medium at high temperature (34° C. to 38° C.) for several days with the addition each day of fresh blood and, after two days, of small amounts of certain bacteria, continues a vigorous growth and shows from day to day a marked pleomorphism. The organisms lose their contractile vacuole and the nuclei assume many of the appearances described as characteristics of the "entameba" group in man. As many as eight nuclei have been found in a trophozoite, and six in a cyst, the usual number so far seen is four in each. In this particular as well as in size and in a "cyclic" change of the karyosome, this species most frequently resembles the pathogenic species described as *Entameba tetragena.* Conclusion: (1) Cultural amebas isolated from the stools of dysenteric patients, are much more complicated in morphology than we have been led to think, and grown under conditions approaching those found in the intestines they closely resemble species described as strict parasites. (2) The question of species and pathogenicity of amebas found in dysenteric stools will probably be settled finally and not until then, when a comparative study is made of amebas in their natural habitat with pure cultures isolated from the same cases and grown *under conditions similar to those found in the habitat from which many were isolated.*

Observation on the Intestinal Bacteria in Pellagra: W. J. MACNEAL.

This report is based upon the work of the Illinois State Pellagra Commission[1] and of the Thompson-McFadden Pellagra Commission of the New York Postgraduate Medical School. A general survey of the fecal bacteria by the methods previously employed in studying the feces of healthy men[2] showed considerable variation from

[1] *Archives of Internal Med.,* August, 1912, pp. 123–168, and September, 1912, pp. 219–249.

[2] *Journ. of Infec. Diseases,* Vol. 6, No. 2, April, 1909, pp. 123–169, and Vol. 6, No. 5, November, 1909, pp. 571–609.

the normal numerical relationships and the advent of new types of bacteria, not observed in healthy men. The most evident change was the relative increase in certain normal types such as *B. bifidus*, *B. welchii* and the micrococci. The cocci were always increased during the acute attack. Other changes were not constant. About 800 bacterial strains were isolated by plate cultures of feces and of intestinal juice obtained through the Einhorn duodenal tube, and these were subjected to agglutination tests with serum from pellagrins at Peoria, Kankakee and Chicago, Ill., and Spartanburg, S. C. One of the bacterial strains is completely agglutinated by the serum of 81 of 109 cases of pellagra (74.3 per cent.), and by 11 of 45 control cases believed to be free from the disease. Similar organisms have been found in the duodenal juice in a few others. The work is being continued.

A Study of Diarrhea in Infants: A. W. Street, Brown University.

This work is a study of the rapid diagnosis of dysentery from the stools of infected infants. It consists of inoculation in special broth tubes from the swabs of the stools, and subsequent isolation of the organism believed to be the cause of diarrhea. We used litmus-lactose-agar and Endo plates and transferred the characteristic growth to other tubes to show cultural characters. In our work we found Russel's medium particularly good for differentiation of the group, and litmus-milk good for differentiation of the two main types. We also used lactose-peptone bile, saccharose and dextrose broth, gelatine, peptone and mannite-litmus semi-solid medium. The incubations were all at 37° C. except gelatine, which was at 20° C., but for varying lengths of time. Generally the incubations were for eighteen hours. Not all the cases were sent to be diagnosed—only the most severe and those reported to the nurses by the physicians. The agglutination test, which is recognized as the most conclusive, was not regularly tried, because of the fact that no good serum was immediately procurable. Agglutination occurs, however, in dilutions of 1:200 and 1:500. Of the cases sent in, which numbered 47, seven showed reactions of those of the dysentery group. They produced acid in litmus milk, and so are of the Flexner type. Many showed reactions in culture tubes very similar to the control tubes, but these failed to check up in one tube or another. So that we are able to conclude that the method of rapid isolation is practical, as is shown in seven of forty-seven cases, or 14.89 per cent.

Bacteriology and Control of Acute Infections in Laboratory Animals: N. S. Ferry, Detroit, Michigan.

Diseases in Epidemic Form Studied: An infection in rabbits, dogs, guinea-pigs and monkeys due to the *B. bronchisepticus*, the microorganism which has been found to be the cause of canine distemper and an infection among rabbits due to the bacillus of rabbit septicemia. Study of Organisms Resembling the *B. bronchisepticus:* During the course of the studies on the epidemic which raged among the several animals ten different organisms were encountered which resembled the *B. bronchisepticus* somewhat in their morphology, their early growth on agar and their behavior toward Gram's stain. A careful study of these organisms showed them to be connected with the epidemic only in the capacity of secondary invaders. Primary Infection: The primary infection was found to be due to the *B. bronchisepticus.* Agglutination tests showed the *B. bronchisepticus* to be absolutely distinct from any of these other organisms. Control of Epidemics: The epidemics were controlled by isolation, antisepsis and the use of prophylactic injection of vaccines made from the specific microorganisms. Epidemic due to the Bacillus of Rabbit Septicemia: After the epidemic due to the *B. bronchisepticus* was under control, an epidemic broke out among the rabbits, due to the bacillus of rabbit septicemia. This epidemic proved very fatal before it could be controlled. The same methods of control were carried out as before. Value of the Protective Inoculation: Although all animals can not be saved by means of the prophylactic injection, control experiments have proved that a large majority are protected.

The Lesions produced by Intra-bronchial Insufflation of B. prodigiosus: Martha Wollstein, M.D., and S. J. Meltzer, M.D.

We inoculated broth cultures of *B. prodigiosus* into the lungs by means of intra-bronchial insufflation, which consists of the introduction of a tube through the mouth, larynx and trachea into a bronchus, and the injection of the fluid culture through the tube. Doses of 5 c.c. to 15 c.c. of a twenty-four-hour broth culture injected into the lungs of dogs were uniformly fatal in six hours to three days, the great majority of animals dying within twenty-four hours. It was not until the dose was reduced to one cubic centimeter that three out of five dogs survived until the fourth day. The entrance of *B. prodigiosus* into the blood stream followed intra-bronchial insufflation

of all doses of one cubic centimeter or more of this organism. The bacillus grew profusely from the heart's blood of every case examined, from five and three fourths hours to four days after inoculation. After the fourth day no growth could be obtained from either heart or lungs. The pulmonary lesions produced by intra-bronchial insufflation of *B. prodigiosus* in dogs differed very markedly from the experimental pneumonias which have hitherto been produced by other bacteria administered in this way. Thus large doses (5 to 15 c.c.) caused pulmonary lesions which were chiefly hemorrhagic and necrotic in character, with a large production of fibrin. Bacteremia and death were the rule. The lung lesions were more severe and the death rate higher than was the case with other bacteria administered intra-bronchially. Very small doses (0.5 c.c.) caused a fibrinous inflammation, lobular at first, later coalesced and lobar in distribution, without evidence of necrosis. No bacteremia followed these small doses and recovery was possible.

Frequency of Vincent's Angina among Routine Throat Cultures: JOHN L. RICE, Syracuse Medical School.

From the examination of 1,352 routine throat swabs 10, or seven tenths of one per cent., were found to be both bacteriologically and clinically Vincent's angina, both the fusiform bacillus and the spirochete being found. Four of the ten cases were clinical cases of diphtheria, showing that 40 per cent. of the Vincent's angina cases were also positive for diphtheria. In twelve other cases of the 1,352 the bacilli or the spirochetes were found alone. None of these twelve cases were clinically Vincent's angina. Morphologically the bacilli in the clinical and non-clinical cases were alike. In a microscopical examination of a smear from the swabs a diagnosis can be made by finding fusiform bacilli and spirochetes in symbiosis, even though the number of spirochetes present may be small.

Studies on the Etiology of Hog Cholera: WALTER E. KING and F. W. BAESLACK, Research Laboratory, Parke, Davis & Co., Detroit, Mich.

This report is presented for the purpose of recording certain observations, which have been made by the aid of the dark field on the blood of hogs suffering from hog cholera. During the last few months a spirochete has been found with uniformity and constancy in the blood of every cholera hog examined. Practically all of these positive findings have been controlled by one or more careful dark field examinations of the blood before inoculation. Additional checks are furnished in several cases by negative findings subsequent to positive results in blood of hogs recovered from the disease. In so far as the present results go, the practised observer can readily distinguish certain characters in the blood of animals suffering from hog cholera when placed on the dark field, as differentiated from normal hog blood. Hog cholera blood usually contains many granules, some very fine yet distinctly larger than blood dust, some larger still, and some very distinct, highly refractive bodies. It is possible that some or all of these granules represent disintegrated blood elements resulting from the disease. It is suggested, however, that some of these granules may represent certain stages in the life cycle of the spirochete under observation. To date, positive findings of this spirochete are recorded in 10 strains of virus from the blood of 33 hogs suffering from the disease. Controls are furnished by negative findings in the blood of about 50 normal hogs and in the blood of six animals which became convalescent and finally recovered. Two experiments have been made relative to the horse serum virus phenomenon, which showed the presence of the spirochete in the horse serum virus.

IMMUNITY BACTERIOLOGY

The Relation of the Leucocytic Bacteriolysin to Body Fluids: W. H. MANWARING, Rockefeller Institute for Medical Research.

A bactericidal substance can be extracted from horse leucocytes. This substance is strongly bacteriolytic when dissolved in distilled water and possesses considerable bactericidal power when dissolved in physiological saline. The substance, however, is without bactericidal properties when mixed with sera, with pathological transudates, with cerebro-spinal fluid, or with the products of tissue autolysis, including the products obtained by a prolonged autolysis of leucocytes themselves. The antibactericidal action of body fluids and tissue products depends upon three factors: (1) the antibactericidal power of the colloids they contain, (2) the antibactericidal power of their neutral salts and other neutral diffusible components and (3) the antibactericidal power of their diffusible alkalies. Diffusible acids are apparently without antibactericidal effect. An extract from horse leucocytes can have little or no antiseptic action, when injected into body cavities and tissue spaces.

On Intraperitoneal Lysis of Tubercle Bacilli: W. H. MANWARING and J. BRONFENBRENNER, Rockefeller Institute for Medical Research.

If suspensions of tubercle bacilli are injected into the peritoneal cavities of tuberculous guinea-pigs, there takes place a rapid disappearance of the bacilli from the peritoneal fluids, as determined by subsequent examinations by the Ziehl-Neelson method. Nine tenths of the bacilli may disappear within an hour, and all but an occasional bacillus within five hours. This disappearance is paralleled by the appearance of atypical, non-staining and granular forms. After the disappearance numerous granules can be demonstrated in the peritoneal fluids and peritoneal scrapings by the Much method. Before the conclusion can be drawn, however, that the disappearance of the tubercle bacilli is due wholly to their destruction by the peritoneal fluids, such factors as a possible removal of the bacilli by the rapid formation and absorption of peritoneal transudate must be ruled out, as well as the possibility of a spontaneous metamorphosis of the bacilli into non-staining and therefore invisible forms, as described by Much. A similar rapid disappearance is brought about in the peritoneal cavities of tuberculous rats, tuberculous rabbits and tuberculous dogs. The mechanism of the disappearance is now under investigation.

The Chemistry of Anaphylactic Intoxication: BENJAMIN WHITE, Hoagland Laboratory, Brooklyn, N. Y.

The study of the chemical problems involved in the anaphylactic phenomenon would seem to offer a promising field. If this reaction is to be considered as a parenteral digestion of protein, then it may be possible to study the reaction *in vitro*. The work of Vaughan on the poisonous substance obtained from proteins by alkali hydrolysis, the work of Biedl and Kraus and others on the action of proteoses and peptones and the experiments of Rosenow on the products of *Pneumococcus* autolysis, appear to be closely related, and these in turn bear resemblances to the results of experiments on the anaphylatoxin produced in the test tube. Recent studies on the action of the amines, particularly that of B-amid-azolylethylamine, suggests a possible analogy between the action of this class of substances and the substances mentioned above.

Peptotoxin Production by the Bacillus of Contagious Abortion in Cattle:[1] JOHN REICHEL, V.M.D., and MALCOLM J. HARKINS, V.M.D.

The English commission appointed by the Board of Agriculture and Fisheries to inquire into epizootic abortion of cattle, in their report include the statement "apparently, however, no free toxins are formed by the bacillus (abortus) in culture." The reaction in infected cattle, usually appreciable by a rise of temperature, etc., in from eight to eighteen hours after a subcutaneous injection of abortin, *i. e.*, an extract of the bacillus and its products prepared as in tuberculin with tubercle bacilli is generally attributed to toxins of which the English commission remarks, "the toxins, then, which cause the febrile symptoms after inoculation are endotoxins, that is to say, they are contained inside the bacilli." From this it may be taken that the opinion is held that the bacilli in culture form no other toxins than endotoxins. From our experiments we have drawn the following conclusions: (1) The bacillus of contagious abortion of cattle (*abortus bacilli*) produces a toxin on peptonized culture media, but not on peptone-free media. (2) Thorough washing will rid the bacilli grown on peptonized media of the toxin. (3) The toxin is included in the alcoholic precipitate of the supernatant liquid of the suspension of the bacilli grown on peptonized agar. (4) Sixty-five degrees centigrade for thirty minutes apparently had no effect on the peptotoxin. (5) Cattle must be sensitized to react to the peptotoxin. (6) *Bacillus typhosus, coli communis,* tetanus and pneumococcus cultures on peptonized agar reveal the presence of peptotoxin, when injected into animals sensitized to the abortus bacillus or its products. The peptotoxins of these organisms probably have much in common if they are not one and the same substance, because animals can be sensitized with one for any of the others. (7) No reactions were observed following the injections into the sensitized animals of peptonized agar cultures of the diphtheria bacillus, *Staphylococcus aureus,* nonhemolytic streptococcus and hemolytic streptococcus which may mean that these organisms did not produce peptotoxin or only in very small amounts. (8) Rabbits developed agglutinins following the injection of thoroughly washed and unwashed abortus bacilli equally well. The peptotoxin injected with the unwashed bacilli is not essential in the production of antibodies. (9) In that the abortus bacillus produces a peptotoxin in a proteid medium—and it is a possibility that the peptotoxin is produced in milk with the bacilli from cattle in infected herds—the wholesomeness of such milk is more than questionable.

[1] The Mulford Laboratories, Glenolden, Pa.

A. PARKER HITCHENS,
Secretary